Summary of data on effects of chemical disinfection (chlorox) and standard autoclaving on SV40 virus and its isolated DNA

I. Treatments

a) Chlorox treatment - 10% commercial chlorox - 6 hours at room temperature and then dialysis overnight against several changes of dialysate.

b) Autoclaving - 20 minutes at 120°C.

II. Experiments with SV40 virus

a) A suspension (0.2 ml) of SV40 virus (8×10^9 pfu/ml) and a 1:10 dilution of that were treated with an equal volume of the chlorox solution for 6 hours at room temperature. Two and one plaque, respectively, were produced in the standard plaque assay on CV-1P monkey kidney cell cultures; mock treated samples gave 6×10^8 and 6×10^7 pfu, respectively.

Thus, chlorox treatment produced approximately 10^8 reduction in infectious titer of SV40 virus.

b) After autoclaving a suspension of SV40 virus (10^9 pfu/ml) no SV40 plaques were produced after infection with 0.2 ml of the undiluted autoclaved solution. No plaques were produced when the same solutions were used to infect monolayers in the presence of DEAE-dextran, a procedure which tests for the residual presence of infective free SV40 DNA.

III. Experiments with SV40 DNA

a) Incubation of a solution of SV40 DNA (6 μg/ml) in 2% chlorox for six hours at room temperature caused the degradation of all the viral DNA to acid-soluble fragments.

b) Autoclaving solutions of SV40 DNA (2.8 μg/ml) in Tris (10^{-2} M)-EDTA (10^{-3} M)(TEN), or TEN with 0.1 M and 1 M NaCl, destroyed completely the infectivity of the DNA measured in the standard plaque assay with DEAE-dextran (no plaques with duplicate 0.2 ml aliquots). Thus, this treatment reduces the infectivity of this covalently closed circular DNA by at least 10^6 fold.

The ability to obtain relatively large quantities of pure segments of DNA from the chromosomes of any living organism will have a profound effect on future research in many areas of biology. The availability of cloned DNA segments permits basic and medical scientists to approach and answer fundamental questions that have heretofore been unanswerable. Three examples (many more could be cited and innumerable others will emerge in time) illustrate how the recombinant DNA methodology is being applied now to problems in chromosome structure and organization, gene expression and viral oncogenesis.

A. The basic mechanisms of gene expression and regulation in eukaryote and, particularly, mammalian and human cells are very poorly understood. One approach which is particularly promising is to isolate mRNAs for particular proteins (e.g., the α and β chains of globin, heavy and light chains of immunoglobulins, histones, con- and ovalbumins etc) and to copy their sequence into unlabeled or isotopically-tagged complementary DNA molecules (cDNAs) using appropriate reverse transcriptases. Such DNA probes can be used to determine the number and chromosomal location of genes that code for the particular mRNA and its protein product; moreover, the cDNA probes permit one to quantify the production of these specific mRNAs in the course of development (embryonic and adult stages), under different environmental conditions (presence and absence of growth factors, hormones and other intercellular signals) and in certain pathologic states.

For example, cDNAs corresponding to the mRNAs coding for the alpha, beta, gamma and delta chains of human hemoglobin, have been used to quantify the number and functional state of these genes in human thallasemic cells (similar experiments could be done for any genetic disease in which a specific protein fails to be made). These experiments indicate that some or all of the genes for a particular hemoglobin protein are absent in each of the different thallasemias. But one problem which limits the application of this approach and the interpretation of the data is the purity of the cDNA probes; of necessity the cDNAs can be only as pure as the mRNA preparations used to direct their synthesis.

Several laboratories (Rougeon et al., Nucleic Acids Res. 2: 2365 (1975); Kafatos et al., Cell, February (1976); Rabbitts, Nature 260: 221 (1976)) have succeeded in cloning the cDNA's produced from isolated mRNAs that code for the hemoglobin polypeptide chains. This procedure provides a relatively simple way to obtain large quantities of homogeneous DNA segments corresponding to particular genes. In short, molecular cloning provides the simplest and most effective means for obtaining pure DNAs. No other procedure, not even chemical synthesis, could provide material of 100% purity. Now these pure DNA probes can be used to obtain more precise evidence on the presence or absence, the organization and the regulation of expression of these genes in various cells and tissues of normal and pathologic physiologic states.

B. A major problem in understanding the mechanism of viral oncogenesis is how and where the infecting or endogenous viral genomes are integrated into the transformed cell's chromosome. This has a bearing on the question of how integrated viral gene expression affects cellular regulation leading to the abnormal growth phenotype characteristic of malignant cells. This problem is central to our understanding of several tumor viruses: the papova viruses, polyoma and SV40; the human adenoviruses, herpes and EB viruses and the RNA sarcoma and leukemia viruses.

The most direct way of examining the structure of the integrated viral DNA sequences is to isolate that segment of the transformed cell's chromosomal DNA containing the viral DNA sequence. But this is a formidable problem. Although the figures vary with the relative amount of DNA in cellular genomes, the "concentration" of viral DNA sequences amongst the cellular DNA sequences is of the order of one part per million for a single integrated SV40 DNA copy per cell, to five parts per million for a single oncorna virus genome per cell and up to fifty parts per million for a single herpes or EB virus genome per cell. Consequently the isolation of the integrated viral DNA segment, free of the bulk of the cellular DNA, from transformed or tumor cells requires a purification of the desired segment of 10^5- or more-fold. Even though restriction enzymes permit the excision of such segments, there is not, at the present time, physical fractionation procedure(s) that can be used to purify such excised segments.

But even if such a methodology existed, there is a considerable logistics problem; prodigous quantities of transformed or tumor cell DNA would be needed as starting material for such an isolation. For example, about 200-300 liters of expensive tissue culture medium ($10/liter) would be needed to prepare sufficient cells to obtain 1 gram of cell DNA containing one or two μg of integrated SV40 DNA (assuming 100% yield); how much medium and cells would it take to work out the isolation procedure? If the viral genome is larger and there are multiple copies of the viral genome per cell, the difficulty is reduced by one to two orders of magnitude.

Molecular cloning reduces the problem to manageable proportions. Only a partial physical purification ($\sim 10^2$-10^3 appears to be possible with several techniques) prior to insertion of this enriched population of DNA segments into a suitable cloning vehicle (plasmid) and its propagation in the appropriate bacterial host. The clones produced in this way can be screened for their content of the desired viral segment by a relatively simple analytical procedure. Thus, it is possible to isolate the segment of interest in pure form; moreover, large enough quantities can be obtained for detailed study by simply extracting a culture of the bacteria carrying the integrated viral DNA segment in its plasmid.

If such isolated DNA's were available, we could determine at which site on the viral DNA integration occurs; is it unique or is it random? We could determine whether certain regions of the integrated viral genome are deleted and whether the host DNA sequences into which the viral DNA has integrated is the same in all transformants, and whether that sequence is relevant to the tumor phenotype.

C. We know little about the organization and function of the genomes in higher organisms. For example, our estimates of the fraction of these genomes that code for proteins are at the level of educated guesses; and we have almost no clues about the function of the noncoding sequences. We suspect that many of these sequences are involved in the regulation of gene expression, but our ignorance about the mechanism of that regulation precludes definitive statements.

This state of ignorance is largely attributable to our previous inability to isolate discrete segments of these genomes in a form that permits detailed molecular analysis. One of the major effects of the advent of the recombinant DNA methodology is to remove this barrier. This methodology provides the means for isolating segments of chromosomal DNA from any organism in large amounts and in homogeneous form. Furthermore, ancillary techniques have been developed whereby one can screen or select for cloned DNA segments that contain particular structural genes (i.e., coding sequences) and/or other sequences of interest.

Because of this extraordinary change in our capacities we are, even now, determining topographies of sequence and function in certain eukaryotic chromosomes at the same level of resolution that was hitherto reserved to viral and plasmid DNAs. For example, cloned DNA segments that contain specific structural genes have been isolated from the chromosomes of Drosophila melanogaster and from sea urchins. The location and orientation of the sequences corresponding to the mRNAs of these genes have been determined, and the mapping of the sequences corresponding to the respective primary transcripts is now in progress. We therefore have at hand the means for locating and characterizing several kinds of genetic signals that are relevant to gene expression - e.g., the sequences present at the sites of initiation and termination of transcription, and the sequences present at the boundaries of the overlap between the mRNA and transcribed sequences, i.e., processing sites. Furthermore, the sequences lying just outside the transcribed regions can also be examined for regulatory functions. Of particular interest in this regard are cloned segments containing genes that respond to steroid hormones, as for example, the genes in D. melanogaster that are known to be induced by ecdysone.

It should also be emphasized that the potential afforded by recombinant DNAs for determining topographies of sequence and function can be extended from maps of individual segments to maps of the entire genome. Radioactive copies of the sequences contained in these segments (e.g., cRNAs transcribed in vitro) have been used as probes to map, by in situ hybridization, all sites within the genome that contain a particular sequence. The many sites occupied by specific repetitive sequences in D. melanogaster have been mapped within the polytene chromosomes in this manner. In one case, a cloned structural gene was shown to be repeated and located at 33 different locations within the genome. In another case, this type of genomic mapping was used to demonstrate that certain repetitive sequence elements in the D. melanogaster are arranged in multi-element blocks that are dispersed throughout the genome, and that the blocks at different sites consist of different, partially overlapping combinations of these elements. This is an intriguing arrangement since it corresponds to that postulated for the repetitive regulatory sequences in the Britten-Davidson model for the control of gene expression in higher organisms. While the applicability of the model to the blocks of repetitive elements in D. melanogaster has yet to be tested,

it is clear that such tests will require the use of recombinant DNAs.

This limited set of examples from existing research projects does not include experiments with DNAs from mammals, as this kind of research has been inhibited by the Asilomar and subsequent guidelines. Although many of the problems illustrated in the above examples have their analogues in mammals, it should be realized that this group provides the best and sometimes the only material for the study of certain areas of gene expression that include most of the medically oriented problems. Of particular interest would be the isolation of cloned DNA segments that contain the variable and constant genes of the immunoglobulins. The analysis of such segments obtained from both germ line and somatic cells should be of inestimable value in determining the mechanism of immunologic diversity.

The ability to clone segments of chromosomal DNA has induced a profound change in our perception of what can be learned about the genomes of higher organisms. Feasible experimental solutions to a very wide range of problems can now be imagined, whereas that range was quite narrow before the advent of recombinant DNAs. In selecting the above examples, I have been deliberately conservative and restricted myself to problems that are being worked on at the moment. Speculation about what could be done in the near future would increase the number of these pages by an order of magnitude. Five years from now we will be astonished at the range of applications and the new insights it has generated.